

**REMARKS*****Status of the claims***

Claims 22-41 and 61-88 were pending in the present application and all claims have been rejected. By virtue of this response, claims 23-30, 32-37, 61, 63-69, 71-75, 79-80, and 84-88 have been cancelled, claims 22, 31, 62, and 70 have been amended, and new claims 89-110 have been added. Accordingly, claims 22, 31, 38-41, 62, 70, 76-78, 81-83, and 89-110 are currently under consideration.

With respect to all amendments and cancelled claims, Applicants have not dedicated or abandoned any unclaimed subject matter and, moreover, have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants reserve the right to pursue prosecution of any presently excluded claim embodiments in future continuation, continuation-in-part and/or divisional applications.

***Amendments to the Claims***

Claims 23-30, 32-37, 61, 63-69, 71-75, 79-80, and 84-88 have been cancelled, claims 22, 31, 62, and 70 have been amended, and new claims 89-110 have been added. No new matter is added.

Claim 22 has been amended, without prejudice, to incorporate the limitations of dependent claims 28, 30, and 32. Claims 22, as amended, is directed to an isolated *Listeria monocytogenes* bacterium which is (a) defective with respect to internalin B, such that the bacterium is attenuated for entry into non-phagocytic cells relative to wild type, and (b) defective with respect to ActA, such that the bacterium is attenuated for cell-to-cell spread relative to wild type, wherein the non-phagocytic cells are hepatocytes. Support for this amendment is found, e.g., in original claims 28, 30, and 32, as well as throughout the specification.

New independent claim 91 is directed to an isolated *Listeria monocytogenes* bacterium which is (a) defective with respect to internalin B, such that the bacterium is attenuated for entry into non-phagocytic cells relative to wild type, wherein the non-phagocytic cells are hepatocytes,

and (b) defective with respect to ActA, such that the bacterium is attenuated for cell-to-cell spread relative to wild type, wherein the bacterium comprises a nucleic acid molecule encoding a non-Listerial antigen. Support for this claim, is found throughout the specification including, but not limited to, paragraphs [0008] and [0009].

New independent claim 102 is directed to a pharmaceutical composition comprising: (1) an amount of *Listeria monocytogenes* bacteria effective to induce an immune response in a human, wherein the bacteria are (a) defective with respect to internalin B, such that the bacteria are attenuated for entry into non-phagocytic cells relative to wild type, and (b) defective with respect to ActA, such that the bacteria are attenuated for cell-to-cell spread relative to wild type, wherein the non-phagocytic cells are hepatocytes; and (2) a pharmaceutically acceptable carrier. Support for this claim, is found, e.g., at paragraphs [0009] and [0043], as well as elsewhere in the application.

Due to the amendment of claim 22, a number of claims have been cancelled. Also, conforming amendments have been made to claims 31, 62, and 70 in light of the amendment of claim 22 and/or the claim cancellations. In addition, typographical errors have been corrected in claims 62 and 70.

Support for new dependent claims 89, 90, 92-101, and 103-110 is found throughout the application as filed and as indicated in the following table:

NEW CLAIM NO(S).	EXEMPLARY SUPPORT IN SPECIFICATION AND/OR ORIGINAL CLAIMS
89, 93	Paragraphs [0009] and [0078]
90	Paragraphs [0009], [0157], and [0202]
92	Paragraphs [0009] and [0041]
94	Paragraph [0159]
95	Paragraph [0160]
96	Paragraphs [0157] and [0163]-[0168]
97	Paragraph [0165]

98, 99	Paragraphs [0008] and [0009]
100	Paragraphs [0008], [0009], and [0043]
101	Paragraphs [0008] and [0009]
103	Paragraphs [0008], [0009], and [0043]
104, 105	Paragraphs [0008] and [0009]
106, 107	Paragraph [0103]
108	Paragraphs [0009], [0184], and [0187]
109, 110	Paragraphs [0008], [0009], [0157], and [0202]

### ***Claim Objections***

The Examiner has indicated that if claims 61-68 are found allowable, claims 69-75 would be objected to under 37 C.F.R. § 1.75 as being a substantial duplicate thereof. The Examiner has recommended deleting claims 69-75.

Applicants respectfully traverse this rejection.

Without acquiescing to the objection and solely in the interest of expediting prosecution, Applicants have cancelled claims 61, 63-69, and 71-75 and have amended claims 62 and 70. The rejection of the cancelled claims is considered moot. Applicants believe that since remaining claim 62, as amended, depends from claim 39, and since remaining claim 70, as amended, depends from claim 40, claims 62 and 70 are not substantial duplicates of each other.

In light of the foregoing, Applicants respectfully request that the objection be withdrawn.

### ***Claim Rejections under 35 U.S.C. § 112, Second Paragraph***

Claims 22-41 and 61-88 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

1. *Claim 22*

The Examiner alleged that “Claim 22 is vague and indefinite because it attempts to claim the bacterium by function alone.”

Applicants respectfully traverse this rejection.

Without acquiescing as to the merits of the rejection and solely in the interest of expediting prosecution, Applicants have amended claim 22 to include the elements of dependent claims 28, 30, and 32. Claim 22 is now directed to a bacterium which is (a) defective with respect to internalin B, such that the bacterium is attenuated for entry into non-phagocytic cells relative to wild type, and (b) defective with respect to ActA, such that the bacterium is attenuated for cell-to-cell spread relative to wild type, wherein the non-phagocytic cells are hepatocytes. Accordingly, the claim now specifies that the attenuation for entry into the hepatocytes is due to the bacterium being defective with respect to a particular protein, the internalin B protein. Likewise, the claim now specifies that the attenuation for cell-to-cell spread is due to the bacterium being defective with respect to a particular protein, the ActA protein.

In light of the foregoing, Applicants respectfully request that the rejection be withdrawn and not be applied to the newly added and amended claims.

2. *Claim 23*

The Examiner has asserted that there is insufficient antecedent basis for the limitation “the mutation” in claim 23. Without acquiescing as to the merits of the rejection and solely in the interest of expediting prosecution, Applicants have cancelled claim 23. Accordingly, this rejection is believed to be moot. Applicants respectfully request that the rejection be withdrawn.

3. *Claim 25*

The Examiner has asserted that claim 25 is unclear whether the reaction causing the modification is the same 'reaction, mutation, etc.' causes the attenuation in claim 22. Without acquiescing as to the merits of the rejection and solely in the interest of expediting prosecution, Applicants have cancelled claim 25. Accordingly, this rejection is believed to be moot. Applicants respectfully request that the rejection be withdrawn.

4. *Claim 27*

The Examiner asserted that claim 27 is vague and indefinite because it is unclear what is encompassed by the phrase "defective with respect to one or more internalins."

Applicants respectfully traverse this rejection.

Although claim 27 has been cancelled by virtue of this amendment, claim 22, as amended, as well as new claims 91 and 102, recite the phrase "defective with respect to internalin B, such that the bacterium is attenuated for entry into nonphagocytic cells relative to wild type...wherein the non-phagocytic cells are hepatocytes." Accordingly, Applicants will focus their arguments regarding this rejection on claim 22, as amended, and new claims 91 and 102.

Applicants contend that the phrase "defective with respect to internalin B, such that the bacterium is attenuated for entry into nonphagocytic cells relative to wild type...wherein the non-phagocytic cells are hepatocytes" would not have been vague or indefinite to one of ordinary skill in the art, especially in light of Applicants' specification. As indicated in Applicants' specification, the internalin B protein directs uptake of the *Listeria* into hepatocytes. See paragraph [0109] in the specification. Bacteria that are "defective with respect to internalin B" would be recognized as either (a) producing decreased amounts of a functional version of the internalin B protein (relative to wild type) or (b) expressing a version of the internalin B protein that is partially or totally nonfunctional (relative to wild type), or both. This is clearly expressed in paragraphs [0096]-[0097] at page 24, [0105] at page 26, and [0110] at page 27. As indicated in paragraph [0111] at page 27,

in some embodiments, the genome comprises one or mutations in *inlB*. In some embodiments, the mutation of *inlB* is in the coding sequence. Paragraph [0113] at page 28. The specification further indicates in paragraph [0113] at page 28, “In alternative embodiments, expression of *inlB* in the mutant strain is inhibited relative to a non-mutant strain. For instance, the genome of the mutant *Listeria* may comprise at least one mutation in *inlB*, where the mutation hinders expression. For instance, the mutation may be in one or more of the control sequences (such as the promoter or ribosome binding region) of *inlB*, so that expression of *inlB* is decreased or eliminated. Alternatively, the mutant *Listeria* may comprise at least one mutation in a gene other than *inlB*, but which nonetheless results in a diminution of the expression levels of internalin B.”

Assays for determining whether a *Listeria* bacterium is “defective with respect to internalin B” would be routine to one skilled in the art. One skilled in the art could readily assess whether expression of *inlB* (the gene encoding internalin B) was decreased relative to wild-type *Listeria* using common biochemical techniques such as RT-PCR, as well as northern blots or western blots. Determination as to whether or not a particular *Listeria* contained mutations in the *inlB* gene such as deletions, frameshift mutations, insertions, and point mutations that would render the gene product less functional could be determined by sequencing. To confirm that a particular mutant *Listeria* strain is, in fact, functionally attenuated for entry into hepatocytes, one of ordinary skill in the art would be able to use *in vitro* and *in vivo* assays taught in the specification and/or otherwise known to those of ordinary skill in the art. The ability to infect hepatocytes can be measured directly *in vitro*. Teachings and examples regarding such *in vitro* assays can be found, e.g., at paragraphs [0089] and [0092] and in Examples 9 and 10 of Applicants’ specification. *In vivo* methods of assaying for a decreased ability to enter non-phagocytic cells are provided in paragraphs [0093] to [0094], as well as in Example 8.

In light of the foregoing, Applicants respectfully request that the rejection be withdrawn and not be applied to the newly added or amended claims.

5. *Claim 30*

The Examiner asserted that claim 30 is vague and indefinite because it is unclear what is encompassed by the phrase “defective with respect to ActA.”

Applicants respectfully traverse this rejection.

Although claim 30 has been cancelled by virtue of this amendment, claim 22, as amended, recites the phrase “defective with respect to ActA, such that the bacterium is attenuated for cell-to-cell spread relative to wild type.” Accordingly, Applicants will focus their arguments regarding this rejection on claim 22, as amended.

Applicants contend that the phrase “defective with respect to ActA, such that the bacterium is attenuated for cell-to-cell spread relative to wild type” would not have been vague or indefinite to one of ordinary skill in the art, especially in light of Applicants’ specification. The ActA protein is known in the art to be essential for cell-to-cell spread. See paragraph [0108] of the specification. Bacteria that are “defective with respect to ActA” would be recognized by one skilled in the art as either (a) producing decreased amounts of a functional version of the ActA protein (relative to wild type) or (b) expressing a version of the ActA protein that is partially or totally nonfunctional (relative to wild type), or both, in the same manner that bacteria that are “defective with respect to internalin B” would be understood as either (a) producing decreased amounts of a functional version of the internalin B protein (relative to wild type) or (b) expressing a version of the internalin B protein that is partially or totally nonfunctional (relative to wild type), or both. (See discussion above regarding the rejection of claim 27.)

Furthermore, one skilled in the art would be readily able to verify that a particular mutant was defective for ActA by using standard technologies known in the art. For instance, one skilled in the art could readily assess whether expression of *actA* (the gene encoding ActA) was decreased relative to wild-type *Listeria* using common biochemical techniques such as RT-PCR, as well as northern blots or western blots. The existence of mutations in the *actA* gene such as deletions, frameshift mutations, insertions, and point mutations can be determined by routine sequencing. In

addition, assays for confirming that a given mutant is, in fact, attenuated for cell-to-cell spread are provided in Applicants' specification and are known to those of ordinary skill in the art. For instance, Applicants' specification provides non-limiting, exemplary *in vitro* assays for assessing whether *Listeria* are attenuated for cell-to-cell spread such as measuring plaque formation on cell monolayers (see, e.g., paragraph [0129] at pages 32 and 33). Assays for assessing the effect of mutations in ActA on actin polymerization *in vitro* and *in vivo* were known in the art as demonstrated in Skoble et al, J. Cell Biology, 150:527-537 (2000).

In light of the foregoing, Applicants respectfully request that the rejection be withdrawn and not be applied to the newly added or amended claims.

6. *Claim 39*

The Examiner asserted that there was insufficient antecedent basis for the limitation "the antigen" in the last line of the claim. Applicants respectfully traverse.

Applicants note that reference is made to "an antigen" in the first line of claim 39. Applicants therefore believe that proper antecedent basis for "the antigen" in the last line of claim 39 already exists. Claim 39 as written indicates that the bacterium comprises the same antigen to which the immune response is generated. If the Examiner believes that "an antigen" in the last line of the claim would be appropriate despite the recitation of "an antigen" in the preamble of the claim, clarification is requested.

In light of the foregoing, Applicants respectfully request that the rejection be withdrawn and not be applied to the newly added or amended claims.

***Double Patenting******1. Application No. 10/883,599***

Claims 22-38 and 83-88 were provisionally rejected on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-122 of copending Application No. 10/883,599. Applicants respectfully traverse.

Claims 21, 23-30, 32-37, 61, 63-69, 71-75, 79-80, and 84-88, and therefore, the rejection of these claims is considered moot.

With respect to the remaining claims, Applicants note that this is a provisional rejection only. Applicants will address this rejection, if maintained, at the appropriate time if conflicting claims are found allowable.

***2. Application No. 10/773,618***

Claims 39, 40 and 61-82 were provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 20-149 of copending Application No. 10/773,618.

Claims 61, 63-69, 71-75, and 79-80 have been cancelled by virtue of this amendment, and therefore, the rejection is considered moot with respect to these claims.

With respect to the remaining claims, Applicants note that this is a provisional rejection only. Applicants will address this rejection, if maintained, at the appropriate time if conflicting claims are found allowable.

***Claim Rejections under 35 U.S.C. § 112 – Enablement***

Claims 22-41 and 61-88 were rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to comply with the enablement requirement. The basis for the Examiner's written description rejection appears to be primarily three-fold:

- A. The specification allegedly does not enable prevention or protection of any disease, nor treatment of cancer or an infectious disease (other than infection by *Listeria monocytogenes*).
- B. The specification allegedly does not enable a number of mutations in *actA* and *inlB* resulting in attenuation for entry into non-phagocytic cells and cell-to-cell spread that is commensurate with the scope of the claims.
- C. The specification allegedly does not enable *actA* and *inlB* mutants that belong to species of *Listeria* other than *L. monocytogenes*.

Applicants respectfully traverse this rejection.

Without acquiescing to the rejection and solely in the interest of expediting prosecution, Applicants have cancelled claims 23-30, 32-37, 61, 63-69, 71-75, 79-80, and 84-88 and amended claims 22, 31, 62, and 70 without prejudice. The rejection is considered moot with respect to the cancelled claims and Applicants will therefore focus only on the patentability of the remaining claims, as amended, as well as the new claims. However, Applicants note that arguments regarding patentability also apply with respect to the cancelled claims and claims 22, 31, 62, and 70 prior to the present amendment.

Independent claim 22, as amended, is directed to an isolated *Listeria monocytogenes* bacterium which is (a) defective with respect to internalin B, such that the bacterium is attenuated for entry into non-phagocytic cells relative to wild type, and (b) defective with respect to ActA, such that the bacterium is attenuated for cell-to-cell spread relative to wild type, wherein the non-phagocytic cells are hepatocytes. New independent claim 91 is directed to an isolated *Listeria monocytogenes* bacterium which is (a) defective with respect to internalin B, such that the bacterium is attenuated for entry into non-phagocytic cells relative to wild type, wherein the non-

phagocytic cells are hepatocytes, and (b) defective with respect to ActA, such that the bacterium is attenuated for cell-to-cell spread relative to wild type, wherein the bacterium comprises a nucleic acid molecule encoding a non-Listerial antigen. New independent claim 102 is directed to a pharmaceutical composition comprising: (1) an amount of *Listeria monocytogenes* bacteria effective to induce an immune response in a human, wherein the bacteria are (a) defective with respect to internalin B, such that the bacteria are attenuated for entry into non-phagocytic cells relative to wild type, and (b) defective with respect to ActA, such that the bacteria are attenuated for cell-to-cell spread relative to wild type, wherein the non-phagocytic cells are hepatocytes; and (2) a pharmaceutically acceptable carrier. All other pending claims depend from claim 22, claim 91, or claim 102, and therefore incorporate the limitations of one of those claims. The method claims are directed to methods of inducing an immune response to an antigen, methods of treating a disease, or methods of providing protection against a disease in a host.

*A. The full scope of the claims directed to methods of inducing an immune response, methods of treating a disease, or methods of providing protection against a disease is enabled.*

Applicants respectfully contend that the full scope of each of the method claims is enabled, including treatment of infectious diseases other than those caused by *Listeria* and cancer, and providing protection against those diseases. Applicants note that methods directed to “providing protection against a disease” as recited in claims 77 and 108, do not necessarily require that 100% protection against the disease be conferred by the method. For example, in some instances, the protection will merely be partial protection, such as protection that merely slows but does not eliminate the onset or progression of the disease. For example, providing enhanced survival in a individual who already has a disease can be a form of providing protection against a disease. Claims that specifically recite “preventing” disease are no longer pending.

Applicants’ own specification provides working examples which demonstrate the ability of an *actAinlB* double deletion mutant strain of *Listeria monocytogenes* to induce an immune response against an antigen, to treat tumors in an *in vivo* mouse model, and to provide protection against cancer in an *in vivo* mouse model. The immunogenicity of an *actAinlB* double deletion mutant

strain of *Listeria monocytogenes* is demonstrated in Applicants' own specification, e.g., in Example 4 (*in vivo* cytotoxicity) and Example 7 (measurement of CD8<sup>+</sup> model antigen-specific T-cells by Intracellular Cytokine Staining (ICS)) in the specification. The ability of an *actAinlB* double deletion mutant strain of *Listeria monocytogenes* to treat tumor-bearing mice is also demonstrated in Example 4 (p. 70-71) and Figures 2A-C of the application. The *actAinlB* mutant *Listeria* used in Example 4 which expressed the antigen AH1-A5, which is a modified antigen related to the endogenous mouse epitope AH-1 (a component of MMTV gp70 protein), was found to decrease the number of lung metastases that developed in the mice in which tumor cells expressing the MMTV gp70 epitope AH1 had been previously implanted (Figure 2A) and to significantly increase survivability of the mice (Figures 2B-C). These results indicate that the *Listeria*-based vaccine was able to break tolerance against an endogenous antigen.

Furthermore, additional data supporting the enablement of the methods of using the claimed compositions to induce immune responses, to treat cancer, and to provide protection against cancer or other disease conditions can be found, e.g., in other publications. For instance, see U.S. Patent Publication No. 2005/0249748. Paragraphs [0612] to [0616] (Example 31D) and Figure 64 of this patent publication provide data indicating that an *actAinlB* double deletion mutant of *Listeria monocytogenes* that had been engineered to express human mesothelin, a tumor antigen, can slow the growth of a tumors in mice that had previously been implanted with tumor cells that expressed human mesothelin. Thus, as articulated in paragraph [0616] of the publication, the mutant *Listeria* was shown to be protective against the progression of cancer in an *in vivo* model. Paragraphs [0603] to [0606] (Example 31B) and Figure 61 of the publication presents results that show the therapeutic efficacy of an *actAinlB* *Listeria* mutant. As shown in Figure 61 of the publication, *Listeria actAinlB* double mutants that expressed human mesothelin were found to prolong survival in tumor-bearing mice where the tumor cells in the mice had been engineered to express human mesothelin. Similarly, the data in paragraphs [0607] to [0609] (Example 31C) and Figure 62 of the specification demonstrates the ability of *Listeria actAinlB* double mutants to reduce the number of lung tumor nodules that develop in mice that had previously been injected with tumor cells that had been engineered to express human mesothelin. Further evidence of the efficacy of *actAinlB* mutants in the treatment of cancer can be found in U.S. Patent Publication No. 2007/0207170 (see, e.g.,

Figures 13-16, 29, 30, 35B, 36 and 38-39). In addition, data demonstrating that administration of *actAinlB* *Listeria* not expressing a heterologous antigen enhanced survival to tumors in the liver generated in the liver via a hemispleen injection model can be found in U.S. Patent Publication No. 2007/0190029. See, e.g., Figures 1A-1C, 1F, and 20.

In addition, evidence of the ability of *actAinlB* mutants to protect in an *in vivo* model against a subsequent challenge by *Listeria* is provided in Table 1 of the Supporting Information available online at [www.pnas.org/cgi/content/full/0406035101/DC1](http://www.pnas.org/cgi/content/full/0406035101/DC1) for the journal article Brockstedt et al., "Listeria-based cancer vaccines that segregate immunogenicity from toxicity," *PNAS*, 101:13832-13837 (2004). Table 1 of the Supporting Information indicates that immunization with 0.1 LD<sub>50</sub> of the *actAinlB* *L. monocytogenes* mutant "CS-L0001," elicits protective immunity (see "Log protection" of Table 1) against subsequent challenge with 2 x LD<sub>50</sub> wild type *L. monocytogenes* 28 days post the primary vaccination.

Thus, it is believed that a variety of data regarding the induction of immune responses providing treatment benefits for and/or protection against a variety of diseases demonstrate that the pending method claims are enabled for their full scope.

Lastly, Applicants draw the following recent review articles to the Examiner's attention as support for the contention that attenuated forms of *Listeria monocytogenes*, in general, have now been recognized in the art as promising antigen-delivery vectors for the treatment and protection of a variety of diseases: Liu et al., "Listeria-Based Anti-Infective Vaccine Strategies," *Recent Patents on Anti-Infective Drug Discovery*, 1:2821-290 (2006); and Bruhn et al., "Listeria as a Vaccine Vector," *Microbes and Infection*, 9: 1226-1235 (2007). Copies of these two review articles are included in the Supplemental Information Disclosure Statement filed herewith.

*B. The full scope of the claims with respect to mutations affecting ActA and internalin B in Listeria monocytogenes is enabled.*

The Examiner has asserted, "Knowledge of the sequence of protein or polynucleotide alone is not sufficient for those skilled in the art to make any mutation to a molecule and have confidence

as to the effects that such a mutation would have.” Later, the Examiner states, “The specification does not provide evidence that one skilled in the art would know what modifications, and what regions of *inlB* and *actA* to target for modifications, in order to produce an attenuated bacterium with the desired phenotype.”

Applicants respectfully traverse and contend that adequate direction and guidance is provided in Applicants’ specification to enable one of ordinary skill in the art to make and/or use the full scope of the claimed invention, including a wide, representative variety of mutants that are defective with respect to ActA and internalin B.

The enablement requirement of 35 U.S.C. § 112, paragraph 1, requires that the claimed invention be enabled so that any person skilled in the art can make and use the invention without undue experimentation. *In re Wands*, 858 F. 2d at 737, 8 USPQ2d at 1404 (Fed. Cir. 1988). The test of enablement is not whether any experimentation is necessary but whether if experimentation is necessary, it is undue. *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976). Furthermore, as stated in MPEP §2164.01, “A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).”

Applicants respectfully submit that the specification, in light of the knowledge of those of ordinary skill in the art at the time, provides a disclosure that is more than adequate to enable one of ordinary skill in the art to make and use a representative number of mutants of *Listeria monocytogenes* that are defective with respect to both ActA and Internalin B.

For instance, Applicants contend that one skilled in the art would be able to recognize or generate a variety of mutants that are defective with respect to the internalin B protein without undue experimentation. Information regarding the sequence of *inlB* in *Listeria monocytogenes* is provided e.g., in paragraph [0221] at pages 61-62, of the present application.

Applicants' specification provides guidance that the defect in internalin B may be effected, e.g., by disruption of the coding sequence (see, e.g., paragraphs [0110] and [0112]) and/or by inhibition of expression of internalin B (see, e.g., paragraphs [0110] and [0113]). As indicated in paragraph [0111] of Applicants specification, a mutation in the *inlB* gene may be a mutation such as a point mutation, an insertion mutation, a termination mutation, a frame shift mutation, or a deletion of part or whole of the gene encoding the internalin B. As indicated in paragraph [0113], in some embodiments, the mutation may be in one or more of the control sequences (such as the promoter or ribosome binding region) of *inlB*, so that expression is deleted or eliminated. One of ordinary skill in the art would be readily able to generate, for example, *Listeria monocytogenes* mutants in which a significant part or all of the *inlB* gene was deleted, mutants in which a stop codon had been placed early in the *inlB* coding sequence, or mutants in which a insertion mutation causes a frame shift in the internalin B coding sequence, all without any undue experimentation. One skilled in the art would further recognize that the *Listeria* could be rendered defective with respect to internalin B by other methods known in the art which don't involve a mutation in the *inlB* gene such as the insertion of an expression cassette in the *Listeria* that expresses an antisense sequence which binds to *inlB*-encoded mRNA.

It is significant that if a mutant of *Listeria monocytogenes* attenuated for entry into hepatocytes is sought, the expression or activity of the *inlB* gene product is being *disrupted* by the mutation(s). Although it may be more of a challenge to identify mutant forms of a protein that maintain function despite the mutations or have improved function due to the mutations, that is not what is required here. Generating functional mutants of a protein can be difficult precisely because it is so easy to render a protein nonfunctional once the sequence is known, even if structural information about the protein is not known. For instance, one skilled in the art will know that a frame-shift mutation is going to disrupt any protein's function, regardless of whether or not the structure-function relationship of the protein is known. Likewise, it is generally routine to eliminate expression of the protein once the gene sequence has been identified. One does not need any special information about the protein such as "which portions of the ... *inlB* gene are necessary for entry into non-phagocytic cells" to know that deletion of the gene, a frame-shift mutation in the coding sequence, or an inserted stop codon early in the coding sequence is going to disrupt

expression. Even many of the possible point mutations that could be generated would be expected to disrupt the production of internalin B, regardless of the specifics of the protein structure. With respect to point mutations, Griffiths et al. states that, "it is always true that such mutations are more likely to reduce or eliminate gene function (thus they are loss-of-function mutations) than to enhance it. The reason is simple: it is much easier to break a machine than to alter the way that it works by randomly changing or removing one of its components." (page 315; Griffiths, et al. (2002) *Modern Genetic Analysis*, W.H. Freeman and Co., New York, NY).

Moreover, making deletions or mutations at any point in a gene and then assaying for loss of function is a well-known method for evaluating regions of a protein that are essential for its function. Such deletions or mutations in a gene can be made using conventional techniques, and the resulting deletions or mutations can be evaluated by methods provided in the specification or otherwise known in the art to assess whether gene function has been sufficiently abrogated.

One skilled in the art would be readily able to verify that a particular mutant was defective for the internalin B protein by using standard technologies known in the art. One skilled in the art could readily assess whether expression of *inlB* (the gene encoding internalin B) was decreased relative to wild-type *Listeria* using common biochemical techniques such as RT-PCR, as well as northern blots or western blots. The existence of mutations in the *inlB* gene such as deletions, frameshift mutations, insertions, and point mutations could be determined by sequencing. To confirm that a particular mutant *Listeria* strain is, in fact, functionally attenuated for entry into hepatocytes, one of ordinary skill in the art would be able to use *in vitro* and *in vivo* assays taught in the specification and/or otherwise known to those of ordinary skill in the art. The ability to infect hepatocytes can be measured directly *in vitro*. Teachings and examples regarding such *in vitro* assays can be found, e.g., at paragraphs [0089] and [0092] and in Examples 9 and 10 of Applicants' specification. *In vivo* methods of assaying for a decreased ability to enter non-phagocytic cells are provided in paragraphs [0093] to [0094], as well as in Example 8. Again, such screening methods do not comprise undue experimentation.

Similarly, Applicants contend that methods for generating *Listeria* that are defective with respect to ActA and attenuated for cell-to-cell spread would also be routine to one of ordinary skill in the art in light of Applicants' disclosure. Information regarding the *actA* gene in *L. monocytogenes* is provided in Applicants' specification. See also Kocks, et al., *Cell*, 68:521-531 (1992) and Genbank accession no. AL591974. Guidance regarding methods of producing the mutants are provided e.g., in paragraphs [0141] to [0142] at pages 38-39 of the specification. As discussed above with respect to *inlB* mutations, one of ordinary skill in the art would be readily able to mutate a target gene so as to disrupt the functionality of its gene product and/or expression of that gene product. For instance, it would be readily apparent to one of ordinary skill in the art that deleting most or all of the gene in question, causing a frame shift mutation, or generating a termination mutation would all be likely to produce the desired effect. In addition, Skoble et al, *J. Cell Biology*, 150:527-537 (2000) describes a variety of *actA* mutants and the effects of such mutations on actin polymerization (an essential function for cell-to-cell spread). One skilled in the art would further recognize that the *Listeria* could be rendered defective with respect to ActA by other methods known in the art which don't involve a mutation in the *actA* gene such as the insertion of an expression cassette in the *Listeria* that expresses an antisense sequence which binds to *actA*-encoded mRNA.

Furthermore, one skilled in the art would be readily able to verify that a particular mutant was defective for ActA by using standard technologies known in the art. For instance, one skilled in the art could readily assess whether expression of *actA* (the gene encoding ActA) was decreased relative to wild-type *Listeria* using common biochemical techniques such as RT-PCR, as well as northern blots or western blots. The existence of mutations in the *actA* gene such as deletions, frameshift mutations, insertions, and point mutations can be determined by routine sequencing. In addition, assays for confirming that a given mutant is, in fact, attenuated for cell-to-cell spread are provided in Applicants' specification and are known to those of ordinary skill in the art. For instance, Applicants' specification provides non-limiting, exemplary *in vitro* assays for assessing whether *Listeria* are attenuated for cell-to-cell spread such as measuring plaque formation on cell monolayers (see, e.g., paragraph [0129] at pages 32 and 33). Assays for assessing the effect of

mutations in ActA on actin polymerization *in vitro* and *in vivo* were known in the art as demonstrated in Skoble et al, J. Cell Biology, 150:527-537 (2000).

Applicants respectfully submit that it would be routine for one of ordinary skill in the art to follow the guidelines set out in Applicants' specification in light of the knowledge of those of ordinary skill in the art to generate a representative number of a variety of mutant *Listeria monocytogenes* that are defective with respect to internalin B and ActA and are therefore attenuated for entry into hepatocytes and for cell-to-cell spread.

C. *The full scope of the claims with respect to Listerial species is enabled.*

The Examiner has asserted, "In the present case, the applicant has neither provided any direction or guidance, nor any working examples in the specification as to any potential mutations of *actA* and *inlB* genes from other species of *Listeria* that would satisfy the limitations of the claims."

Applicants respectfully disagree with the Examiner's assertion.

Nevertheless, without acquiescing as to the merits of the rejection and solely in the interest of expediting prosecution, claim 22 has now been amended, without prejudice, to indicate that the *Listeria* bacterium is a *Listeria monocytogenes* bacterium. New independent claims 91 and 102 are likewise specific to the species of *Listeria monocytogenes*.

In light of the foregoing, Applicants respectfully request that the rejection under 35 U.S.C. § 112, first paragraph as failing to comply with the enablement requirement be withdrawn and not be applied to the newly added or amended claims.

***Claim Rejections under 35 U.S.C. § 112 – Written Description***

Claims 22-41 and 61-88 stand rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to comply with the written description requirement. The primary basis for the Examiner's written description rejection appears to be two-fold:

- A. The specification allegedly does not provide adequate written description to support species homologs to *L. monocytogenes actA* and *inlB* genes.
- B. The specification allegedly does not provide adequate written description to support the scope of mutations.

Applicants respectfully traverse this rejection.

Without acquiescing to the rejection and solely in the interest of expediting prosecution, Applicants have cancelled claims 23-30, 32-37, 61, 63-69, 71-75, 79-80, and 84-88 and amended claims 22, 31, 62, and 70 without prejudice. The rejection is considered moot with respect to the cancelled claims and Applicants will therefore focus only on the patentability of the remaining claims, as amended, as well as the new claims. However, Applicants note that arguments regarding patentability also apply with respect to the cancelled claims and claims 22, 31, 62, and 70 prior to the present amendment.

- A. *The application provides adequate written description to support the full scope of the claims with respect to the species of Listeria recited in the claims as amended.*

The Examiner has alleged that "the specification does not provide adequate written description to support...species homologs to *L. monocytogenes actA* and *inlB* genes."

Applicants respectfully disagree with the Examiner's assertions.

Nevertheless, without acquiescing as to the merits of the rejection and solely in the interest of expediting prosecution, claim 22 has been amended, without prejudice, to specify that the *Listeria* bacterium is a *Listeria monocytogenes* bacterium. New independent claims 91 and 102 are

likewise specific to the species of *Listeria monocytogenes*. Accordingly, support for species homologs to *L. monocytogenes actA* and *inlB* genes should no longer be required.

*B. The application provides adequate written description to support the full scope of the claims with respect to the attenuating mutations.*

The Examiner also states that the “specification does not provide adequate written description to support...any mutation resulting in attenuation for entry into non-phagocytic cells and cell-to-cell spread.” The Examiner asserts that the Federal Circuit has held that claiming polynucleotides disclosed by their biological function alone is inadequate to meet the written description requirement, citing *Amgen, Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991) and *Regents of the Univ. of Cal. v. Eli Lilly and Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997), and that the Federal Circuit case law supports her position regarding Applicants’ claims. More specifically, the Examiner further asserts that the “specification does not provide evidence that one skilled in the art would know what modifications, and what regions of the *inlB* and *actA* coding regions to target for modifications, in order to produce an attenuated bacterium.” The Examiner also states that “one skilled in the art would not be able to recognize from the current disclosure any substitutions, or other mutation (except, perhaps, deletion of the whole polynucleotide) that would result in a decreased gene product activity.” The Examiner cites the reference, Bowie et al., in support of her assertions.

Applicants respectfully traverse this rejection.

It is well established that to meet the written description requirement, an applicant’s specification must “convey to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention.” *Vas-Cath, Inc. v. Marhurkar*, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). This is the standard that the Federal Circuit has set. Applicants contend that the portions of both of the opinions, *Amgen* and *Eli Lilly*, that were cited by the Examiner concerned claims directed to polynucleotides where the sequences of a representative number of the claimed polynucleotides were not previously known in the art. In a subsequent Federal Circuit opinion, *Falkner v. Inglis*, which concerned claims directed to vaccines comprising

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mutant viral sequences, claims that are more analogous to those of the present application than the claims that were the subject of either *Amgen* or *Eli Lilly*, the Federal Circuit clearly stated that “(1) examples are not necessary to support the adequacy of a written description (2) the written description standard may be met...even where actual reduction to practice of an invention is absent; and (3) *there is no per se rule that an adequate written description of an invention that involves a biological macromolecule must contain a recitation of a known structure.*” (emphasis added) 448 F.3d 1357, 1366, 79 U.S.P.Q.2d 1001, 1007 (Fed. Cir. 2006). See also, e.g., MPEP 2163(II)(A)(3)(a). In addition, as indicated in MPEP 2163(II)(A)(2), generally “there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the specification. See, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986).” Furthermore, in *Falkner v. Inglis*, the Federal Circuit held that “where...accessible literature sources clearly provided as of the relevant date, genes and their nucleotide sequences..., satisfaction of the written description requirement does not require either the recitation or incorporation by reference...of such genes and sequences.” 448 F.3d 1357, 1368 (Fed. Cir. 2006).

Applicants further contend that the Bowie et al. reference also does not support the Examiner’s assertions and is largely irrelevant to the present application. The Bowie et al. reference describes “how an analysis of allowed amino acid substitutions in proteins can be used to reduce the complexity of sequences and reveal important aspects of structure and function.” See first paragraph on page 1306 of Bowie et al. The Examiner has pointed to nothing in the reference which would indicate that one of ordinary skill in the art would not readily envision multiple different ways that could be used to *disrupt* the expression or functionality of a given sequence. Even if it is true that one of ordinary skill in the art may often have trouble making amino acid substitutions in a particular protein sequence while still maintaining functionality, this is irrelevant to the present application. Unpredictability in making amino acid substitutions in a protein in which the structure-function relationship is unclear does not translate into there being unpredictability in the ability to *disrupt* the expression or function of a sequence. Regardless of how mysterious the structure-function relationships are, it would be obvious to one of ordinary skill in the art that

disruption of expression and/or function of a gene would most likely occur if certain things are done, such as, but not limited to, any of the following: (a) deletion of the entire coding sequence; (b) deletion of the majority of the coding sequence; (c) generation of one or more stop codons early in the coding sequence; (d) a deletion early in the coding sequence that generates a frame-shift mutation (e) an insertion early in the coding sequence that generates a frame-shift mutation; (f) deletion of the promoter or other key control sequence; and (g) deletion of both the promoter and the coding sequence of the gene. The effect of these types of mutations is far more predictable than the effect of the types of individual amino acid substitutions such as those discussed in Bowie et al.

Moreover, making deletions or mutations at any point in a gene and then assaying for loss of function is a well-known method for evaluating regions of a protein that are essential for its function. Such deletions or mutations in a gene can be made using conventional techniques, and the resulting deletions or mutations can be evaluated by methods provided in the specification or otherwise known in the art to assess whether gene function has been sufficiently abrogated.

Even if single point mutations in a gene are being made, one skilled in the art would recognize that such mutations would be more likely to disrupt the function of the gene than not. As noted above, with respect to point mutations, Griffiths et al. states that, "it is always true that such mutations are more likely to reduce or eliminate gene function (thus they are loss-of-function mutations) than to enhance it. The reason is simple: it is much easier to break a machine than to alter the way that it works by randomly changing or removing one of its components." (page 315; Griffiths, et al. (2002) *Modern Genetic Analysis*, W.H. Freeman and Co., New York, NY).

Applicants contend that, contrary to the Examiner's assertions, sufficient disclosure is provided in Applicants' specification to show possession of a range of mutations having the desired claimed effect of rendering the bacterium defective with respect to the internalin B protein such that the *Listeria* are attenuated for entry into hepatocytes. One of ordinary skill in the art would readily recognize that the inventors were in possession of such a genus of mutations because the specification teaches that altering the strain such that it is defective with respect to internalin B may be effected, e.g., by disruption of the coding sequence (see, e.g., paragraphs [0110] and [0112]) and/or by inhibition of expression of internalin B (see, e.g., paragraphs [0110] and [0113]). As

indicated in paragraph [0111] of Applicants specification, a mutation in the *inlB* gene may be a mutation such as a point mutation, an insertion mutation, a termination mutation, a frame shift mutation, or a deletion of part or whole of the gene encoding the internalin B. As indicated in paragraph [0113], in some embodiments, the mutation may be in one or more of the control sequences (such as the promoter or ribosome binding region) of *inlB*, so that expression is deleted or eliminated. Based on the sequence information taught about *inlB* (see, e.g., paragraph [0109] at page 27), one of ordinary skill in the art would view the inventors as being in possession of, e.g., mutants in which a significant part or all of the *inlB* gene was deleted, mutants in which a stop codon had been placed early in the *inlB* coding sequence, mutants in which an insertion mutation causes a frame shift in the internalin B coding sequence, as well as other types of mutants.

One of ordinary skill in the art would also recognize that the existence of a particular mutation and/or confirmation of the desired effect of a particular mutation could be readily be achieved using one of the *in vitro* and *in vivo* assays taught in the specification and/or otherwise known to those of ordinary skill in the art. One skilled in the art would be readily able to verify that a particular mutant was defective for the internalin B protein by using standard technologies known in the art. One skilled in the art could readily assess whether expression of *inlB* (the gene encoding internalin B) was decreased relative to wild-type *Listeria* using common biochemical techniques such as RT-PCR, as well as northern blots or western blots. The existence of mutations in the *inlB* gene such as deletions, frameshift mutations, insertions, and point mutations could be determined by sequencing. The ability to infect hepatocytes can be measured directly *in vitro*. Teachings and examples regarding such *in vitro* assays can be found, e.g., at paragraphs [0089] and [0092] and in Examples 9 and 10 of Applicants' specification. *In vivo* methods of assaying for a decreased ability to enter non-phagocytic cells are provided in paragraphs [0093] to [0094], as well as in Example 8.

Similarly, one of ordinary skill in the art would readily recognize that the inventors were in possession of a representative number of mutations that would render the bacterium defective with respect to the ActA protein such that the *Listeria* are attenuated for cell-to-cell spread. Guidance regarding methods of producing the mutants are provided e.g., in paragraphs [0141] to [0142] at pages 38-39 of the specification. As discussed above with respect to *inlB* mutations, mutation of a

gene such as *actA* so as to disrupt the functionality of its gene product and/or expression of that gene product would be routine. For instance, it would be readily apparent to one of ordinary skill in the art that deleting most or all of the *actA* gene, causing a frame shift mutation in the *actA* gene, or generating a termination mutation in the *actA* gene would all be likely to produce the desired effect. In addition, Skoble et al, J. Cell Biology, 150:527-537 (2000) describes a variety of ActA mutants and the effects of such mutations on actin polymerization (a function important for cell-to-cell spread).

Furthermore, as already noted above, one skilled in the art would be readily able to verify that a particular mutant was defective for ActA by using standard technologies known in the art. For instance, one skilled in the art could readily assess whether expression of *actA* (the gene encoding ActA) was decreased relative to wild-type *Listeria* using common techniques such as RT-PCR, as well as northern blots or western blots. The existence of mutations in the *actA* gene such as deletions, frameshift mutations, insertions, and point mutations can be determined by routine sequencing. Assays for confirming that a given mutant is, in fact, attenuated for cell-to-cell spread are also provided in Applicants' specification and are known to those of ordinary skill in the art. For instance, Applicants' specification provides non-limiting, exemplary *in vitro* assays for assessing whether *Listeria* are attenuated for cell-to-cell spread such as measuring plaque formation on cell monolayers (see, e.g., paragraph [0129] at pages 32 and 33). Assays for assessing the effect of mutations in ActA on actin polymerization *in vitro* and *in vivo* were known in the art as demonstrated in Skoble et al, J. Cell Biology, 150:527-537 (2000).

In light of foregoing, Applicants respectfully request that the rejection of the claims under 35 U.S.C. § 112, first paragraph as failing to comply with the written description requirement be withdrawn and not be applied to the newly added or amended claims.

***Claim Rejections under 35 U.S.C. § 102***

1. *Appelberg et al. (Infect. Immun. Feb. 2000 68(2): 912-914)*

Claims 22-24, 27-30, 32, 37, 38, and 39 are rejected under 35 U.S.C. § 102(b) as being anticipated by Appelberg et al. (Infect. Immun. Feb. 2000 68(2): 912-914).

Applicants respectfully traverse this rejection.

Without acquiescing to the rejection and solely in the interest of expediting prosecution, Applicants have cancelled claims 23-30, 32, and 37 and amended claim 22, without prejudice. The rejection is considered moot with respect to the cancelled claims and Applicants will therefore focus only on points of distinction with respect to the remaining claims, as amended, as well as the new claims. However, Applicants note that points of distinction also apply with respect to the cancelled claims and claim 22 prior to the present amendment.

Applicants contend that Appelberg et al. does not anticipate independent claim 22 (or new independent claims 91 and 102) because it does not teach a bacterium which is both (a) defective with respect to internalin B, such that the bacterium is attenuated for entry into non-phagocytic cells relative to wild type, and (b) defective with respect to ActA, such that the bacterium is attenuated for cell-to-cell spread relative to wild type, wherein the non-phagocytic cells are hepatocytes. Furthermore, with respect to new independent claim 91, Appelberg et al. further fails to anticipate the claim because it fails to teach a *Listeria monocytogenes* bacterium comprising a non-Listerial antigen. The cited reference also fails to teach a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an amount of the *Listeria* sufficient to induce an immune response in a human, (claim 102), such as an immune response to a non-Listerial antigen (claim 103).

In light of the foregoing, Applicants respectfully request that the rejection under 35 USC § 102 over Appelberg et al. be withdrawn and not be applied to the newly added or amended claims.

2. *Drevets (Infect. Immun. Jan. 1998 66(1): 232-238)*

Claims 22-24, 27-30, 32, 37, 38 and 39 are rejected under 35 U.S.C. § 102(b) as being anticipated by Drevets (Infect. Immun. Jan. 1998 66(1): 232-238).

Applicants respectfully traverse this rejection.

Without acquiescing to the rejection and solely in the interest of expediting prosecution, Applicants have cancelled claims 23-30, 32, and 37 and amended claim 22, without prejudice. The rejection is considered moot with respect to the cancelled claims and Applicants will therefore focus only on points of distinction with respect to the remaining claims, as amended, as well as the new claims. However, Applicants note that points of distinction also apply with respect to the cancelled claims and claim 22 prior to the present amendment.

Applicants contend that Drevets does not anticipate independent claim 22 (or new independent claims 91 and 102) because it does not teach a bacterium which is both (a) defective with respect to internalin B, such that the bacterium is attenuated for entry into non-phagocytic cells relative to wild type, and (b) defective with respect to ActA, such that the bacterium is attenuated for cell-to-cell spread relative to wild type, wherein the non-phagocytic cells are hepatocytes. Furthermore, with respect to new independent claim 91, Drevets further fails to anticipate the claim because it fails to teach a *Listeria monocytogenes* bacterium comprising a non-Listerial antigen. The cited reference also fails to teach a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an amount of the *Listeria* sufficient to induce an immune response in a human, (claim 102), such as an immune response to a non-Listerial antigen (claim 103).

In light of the foregoing remarks, Applicants respectfully request that the rejection under 35 USC § 102 over Drevets be withdrawn and not be applied to the newly added and amended claims.

***Claim Rejection under 35 U.S.C. § 103***

Claim 31 has been rejected under 35 U.S.C. § 103(a) for allegedly being unpatentable over Appelberg et al. (Infect. Immun. Feb. 2000 68(2): 912-914). The Examiner has cited a portion of Appelberg et al. that speculates, that “it will be interesting to analyze the characteristics of double mutants defective in both the ActA and the internalin pathways.” The Examiner further alleged that “one of ordinary skill in the art would have been motivated to produce a mutant deficient in both ActA and *inlB* in order to find a way of treating such a virulent infection.”

Applicants respectfully traverse this rejection.

Claim 31 remains directed to a *Listeria* bacterium which comprises at least one mutation in both *actA* and *inlB*, wherein the mutation in *actA* attenuates the bacterium for cell-to-cell spread relative to wild type and the mutation in *inlB* attenuates the bacterium for entry into hepatocytes relative to wild type.

In response to the Examiner’s assertions, Applicants contend that the claimed bacteria are not obvious over the Appelberg et al. reference, because the claimed bacteria have unexpected, superior properties which one of ordinary skill in the art would not have predicted, even in view of the Appelberg et al. reference. First, Applicants contend that an *actAinlB* double deletion mutant ( $\Delta actA \Delta inlB$ ) provides a level of safety in terms of liver damage that is superior to that of many other attenuated *Listeria* mutants. Second, Applicants have also determined that the  $\Delta actA \Delta inlB$  *Listeria* mutant, despite its superior liver safety profile, surprisingly still possesses the ability to generate robust immune responses, such as CD8+ T-cell immune responses against non-Listerial antigens, that can be superior to those generated by the highly virulent wild-type *Listeria*, as well as to those generated by many attenuated forms of *Listeria*. Those skilled in the art would have been unable to predict that an attenuated *Listeria* mutant that expressed such a favorable liver safety profile would also have such superior immunogenicity. This unexpected and unique combination of superior safety and superior immunogenicity is extremely beneficial in *Listeria*-based immunotherapeutics and vaccines. As noted in the first column of page 13832 of Brockstedt et al., “*Listeria*-based cancer vaccines that segregate immunogenicity from toxicity,” *PNAS*, 101:13832-pa-1241905

13837 (2004), “the practical utility of live attenuated vaccines relies on achieving a proper balance between the virulence/toxicity and immunogenicity of the vaccine.” A useful vector for immunotherapy must combine acceptable toxicity and immunogenicity parameters.

Evidence that an  $\Delta actA \Delta inlB$  mutant exhibits a superior liver safety profile is found in the application as filed. For example, Figure 7A of the present application shows the *in vivo* growth kinetics in liver (a primary site of infection for *Listeria monocytogenes*) for wild type *Listeria monocytogenes* as well as for three mutants:  $\Delta actA$ ,  $\Delta inlB$ , and  $\Delta actA \Delta inlB$ . These data show that the  $\Delta actA \Delta inlB$  mutant was cleared from the liver substantially faster than the wild-type *Listeria monocytogenes*,  $\Delta actA$  mutant, or  $\Delta inlB$  mutant, even though the  $\Delta actA \Delta inlB$  mutant was administered at a 200-fold higher dose than the wild type *Listeria* and the  $\Delta inlB$  mutant. See Figure 7A and paragraphs [0249]-[0251] at pages 75-76. The level of the  $\Delta actA \Delta inlB$  mutant in the liver reached the minimum level by days 3-4. By contrast, the level of the wild type *Listeria* in the liver decreased to the minimum level only by day 11, even though administered at a 200-fold lower dose than the  $\Delta actA \Delta inlB$  mutant. Similarly, the level of the  $\Delta inlB$  mutant, despite being administered at a 200-fold lower dose than the  $\Delta actA \Delta inlB$  mutant, also did not reach minimum levels until by at least day 11. The  $\Delta actA$  mutant, which was administered at the same dose as the  $\Delta actA \Delta inlB$  mutant, nevertheless also failed to clear to minimum levels for at least 7 days and showed significant increases in levels initially relative to the  $\Delta actA \Delta inlB$  mutant as well as the other *Listeria* tested. The superiority of the  $\Delta actA \Delta inlB$  mutant over the  $\Delta actA$  mutant in terms of liver safety is evident from this data, despite the fact that the  $\Delta actA$  mutant and the  $\Delta actA \Delta inlB$  mutant have comparable LD<sub>50</sub> values. (See Table 1 and paragraph [0238] of Example 2 at pages 66-67 of the application, which indicate that the LD<sub>50</sub> of both the  $\Delta actA$  mutant and  $\Delta actA \Delta inlB$  mutant was determined to be approximately  $1 \times 10^8$  colony forming units (cfu)) Furthermore, the effect of the combination of the  $\Delta actA$  and  $\Delta inlB$  mutations on the rate of clearance from the liver in Figure 7A is shown to be synergistic relative to the effects of the single mutations individually on clearance rates.

The superior liver safety profile of the  $\Delta actA \Delta inlB$  mutant relative to wild type *Listeria* and the  $\Delta actA$  and  $\Delta inlB$  mutants is further demonstrated by the data on the clearance rates from liver at

the different set of doses presented in Figure 7B of the application. As shown in Figure 7B, the  $\Delta actA \Delta inlB$  mutant cleared from the liver faster than the  $\Delta actA$  mutant even though it was administered at a 10-fold higher dose than the  $\Delta actA$  mutant. Furthermore, in Figure 7B the  $\Delta actA \Delta inlB$  mutant was shown to clear from the liver at a significantly faster level than the wild type or  $\Delta inlB$  mutant, even though the  $\Delta actA \Delta inlB$  mutant was administered at a dose 2000-fold higher than that of the wild type and  $\Delta inlB$  mutant.

In addition, the safety benefits of the  $\Delta actA \Delta inlB$  mutant are further demonstrated by the data on the clearance of wild type *Listeria* and the  $\Delta actA \Delta inlB$ ,  $\Delta actA$  and  $\Delta inlB$  mutants from the spleen that are presented in Figures 8A and B of the application. As shown in Figures 8A and 8B, the  $\Delta actA \Delta inlB$  mutant was found to clear the spleen at least as fast as the  $\Delta actA$  mutant even though the double mutant was administered at 10-fold higher doses than the single mutant, and also was found to clear the spleen a number of days more quickly than either the wild type *Listeria* or the  $\Delta inlB$  mutant.

Further confirmation of the superior liver safety profile of the  $\Delta actA \Delta inlB$  mutant can be found in a subsequent publication on which Applicants are listed as co-authors, Brockstedt et al., *PNAS*, 101:13832-13837 (2004), including the "Supporting Information" available for the reference online at [www.pnas.org/cgi/content/full/0406035101/DC1](http://www.pnas.org/cgi/content/full/0406035101/DC1). The data in this journal article demonstrate that not only does the  $\Delta actA \Delta inlB$  mutant exhibit superior clearance profiles, but also that lower liver enzyme levels are found in serum following vaccination by the  $\Delta actA \Delta inlB$  mutant than by the  $\Delta actA$  strain. The liver enzyme levels in serum following vaccination with different mutants were tested and  $\Delta actA$ , but not  $\Delta actA \Delta inlB$ , was found to result in significant levels of liver enzymes, such as aspartate aminotransferase (AST) or alanine aminotransferase (ALT), in serum 24 hours after infection. See Figure 6B, of the "Supporting Information" and page 13835 (left column) of Brockstedt et al. (2004). Liver enzyme levels are a known indicator of liver function and abnormally elevated levels of liver enzymes are a known indicator of liver damage. If a strain of *Listeria* induces significant liver damage as, e.g., evidenced by significantly elevated liver enzyme levels, then the liver damage will be dose limiting in clinical practice (thereby limiting the immunogenic benefit that can be derived from the *Listeria*) or potentially will even prevent its use

in humans altogether. Thus, the data in Figure 6B indicates that even if an  $\Delta actA$  mutant has an LD<sub>50</sub> comparable to that of an  $\Delta actA\Delta inlB$  mutant, the  $\Delta actA\Delta inlB$  mutant still provides a liver safety profile that is superior to that provided by the  $\Delta actA$  mutant.

In addition, the histopathology of livers from mice treated with mutants of *Listeria* further indicates that the  $\Delta actA\Delta inlB$  mutant provides a safety profile that is superior to the  $\Delta actA$  mutant in terms of liver safety, despite the similar LD<sub>50</sub> values. Brockstedt et al. (2004) states, “Moreover, histopathological changes in the liver throughout the course of infection were significantly milder in mice receiving the  $\Delta actA/\Delta inlB$  double mutant when compared with wild type-,  $\Delta actA$ -, or  $\Delta inlB$ -infected mice (data not shown).” Page 13835 (left column).

Even more unexpected than the superior liver safety profile is the fact that such an avirulent strain could, despite its superior liver safety profile and high LD<sub>50</sub>, still induce an immune response more potent than wild type *Listeria* and more potent than a wide variety of other mutants in which one or two virulence genes had been mutated. One skilled in the art will recognize that in many instances, mutant strains of *Listeria* that are attenuated may nevertheless not be desirable or suitable for use in immunotherapeutics. For example, the deletion or mutation of a virulence gene may raise the LD<sub>50</sub>, but the resulting attenuated strain may in some cases not be sufficiently immunogenic to be desirable for use in a vaccine. Even if it is possible to administer higher doses of an attenuated strain than wild type safely, the attenuated strain may in many instances nevertheless fail to generate any more significant of an immune response than wild type and may even generate a lower immune response than wild type.

Examples of the fact that different attenuated *Listeria* will have different levels of immunogenicity are readily apparent from the data shown in Table 1 of the “Supporting Information” for Brockstedt et al. (2004) which lists both LD<sub>50</sub> values (see “Pathogenicity in C57BL/6 mice LD<sub>50</sub>” and “Pathogenicity in Balb/c mice LD<sub>50</sub>”) and some measures of immunogenicity (see “OVA-specific CD8+ T cells, %” and “Log protection”) for wild type *Listeria*, heat killed *Listeria*, and a variety of single and double mutants. The column labeled “OVA-specific CD8+ T cells, %” in Table 1 indicates the percent OVA-specific CD8+ T cells generated by the indicated strain of *Listeria*, as determined by ICS, following immunization of mice with a dose

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equal to 0.1 LD<sub>50</sub> of the indicated *Listeria* OVA strain. Of the eleven live attenuated *Listeria* mutants in Table 1 that have higher LD<sub>50</sub> values than wild type, only three proved to be significantly superior at inducing OVA-specific CD8+ T-cells responses than wild type when administered at a dose equivalent to 0.1 LD<sub>50</sub>. Similarly, only four of the eleven mutant *Listeria* were as effective as wild type at providing protection against subsequent challenge with wild type *Listeria* when administered at a dose equivalent to 0.1 LD<sub>50</sub> (see “Log protection” column).

Furthermore, the data in Table 1 of the “Supporting Information” of Brockstedt et al. (2004) also indicate that the  $\Delta actA \Delta inlB$  mutant can generate immune responses that are superior to wild type and most of the other mutants listed. The data in Table 1 indicate that the  $\Delta actA \Delta inlB$  mutant is superior at inducing OVA-specific CD8+ T cell immune responses relative to wild type and to at least ten of the other twelve mutants listed in Table 1. Table 1 also shows that the immunization with the  $\Delta actA \Delta inlB$  mutant at a dose equivalent to 0.1 LD<sub>50</sub> provides maximum protection against subsequent challenge with *Listeria*. In the column labeled “Log protection,” the level of protection afforded by the  $\Delta actA \Delta inlB$  mutant is shown to be the maximum level detectable in the experiment (i.e., 4.86 log protection). At least nine of the other twelve mutants listed in Table 1 were less effective at protection against challenge with wild type *Listeria*.

Evidence that an  $\Delta actA \Delta inlB$  mutant can exhibit a robust ability to generate CD8+ T-cell immune responses against non-listerial antigens that is superior to the highly virulent wild-type and to many attenuated forms of *Listeria*, including many double mutants, is also found in Applicants’ specification. For example, Applicants have tested the immunogenicity of a variety of different mutant forms of *Listeria*. Figure 3A and 3B of the present application show that an  $\Delta actA \Delta inlB$  mutant was found to generate an antigen-specific effector CD8+ T-cell response that was superior to wild-type, most single mutants, and each of the other double mutants tested, when the *Listeria* were administered to the mice at doses of 0.1 LD<sub>50</sub>. See paragraphs [0244] and [0245]. For instance, Figure 3A, shows the results of an IFN- $\gamma$  ICS assay for splenic CD8+ T cells from mice vaccinated with 0.1 LD<sub>50</sub> of the indicated *Listeria*, stimulated with the SL8 OVA<sub>257-264</sub> peptide. These data show that the  $\Delta actA \Delta inlB$  mutant expressing a model antigen, ovalbumin (OVA), generated more robust OVA-specific CD8+ T cell responses than wild-type and all of the other double mutants

tested. The OVA-expressing  $\Delta actA \Delta inlB$  mutant is also shown to induce an OVA-specific CD8+ T cell immune response that is higher than each of the single mutants tested other than L461T and  $\Delta actA$  mutants. The  $\Delta inlA \Delta inlB$ ,  $\Delta inlA$ , and  $\Delta inlB$  were inferior to the  $\Delta actA \Delta inlB$  mutant in their ability to generate an OVA-specific CD8+ T cell immune response in the assay. Figure 3B shows the similar results obtained from a TNF- $\alpha$  ICS assay for splenic T cells from mice. As was the case with the data shown in Figure 3A, the  $\Delta actA \Delta inlB$  mutant expressing OVA is again shown to be superior at generating OVA-specific CD8+ T-cell immune responses relative to wild type *Listeria* and most of the mutants tested.

Figure 4 and paragraph [0246] of the application provides further data supporting the immunogenic superiority of the  $\Delta actA \Delta inlB$  mutant. Figure 4 shows the results of IFN- $\gamma$  ICS assays for spleen cells from mice vaccinated (intravenously) with mutant *Listeria* that have been stimulated with an OVA epitope (SL8) or live or inactivated OVA-expressing tumor cells (EG7 and EG7 PCT). The OVA-expressing  $\Delta actA \Delta inlB$  mutant was found to generated a more significant immune response in this assay than wild-type or any of the other *Listeria* tested including wild type,  $\Delta actA$ , and two other  $\Delta actA$  double mutants.

Applicants contend that one skilled in the art would have been unable to predict on the basis of Appelberg et al. that the  $\Delta actA \Delta inlB$  mutant or other *Listeria* defective with respect to ActA and internalin B would have the superior liver safety profile that Applicants have demonstrated it to have (e.g., exceptional rate of clearance from the liver, low levels of liver enzymes, etc.). Furthermore, even *if* one skilled in the art could predict the favorable safety profile, one skilled in the art would not, as the Examiner suggests, necessarily be inclined to use the mutant *Listeria* as an immunotherapeutic. One skilled in the art would recognize that just because *Listeria* are attenuated does not necessarily mean that the *Listeria* are suitable for use in immunotherapeutics. The Appelberg et al. reference says nothing about the immunogenicity of *Listeria* that comprise mutations in both *actA* and *inlB* or are otherwise defective with respect to ActA and internalin B. Accordingly, Applicants contend that one of ordinary skill in the art would not necessarily be motivated to use the mutant *Listeria* in immunotherapy against Listeriosis, cancer, or any other disease merely upon reading the Appelberg et al. reference. Only Applicants' application teaches

that *Listeria* comprising attenuating mutations in the *actA* and *inlB* genes, or otherwise defective with respect to both ActA and internalin B, can be both particularly safe and particularly potent as immunotherapeutics.

In light of the foregoing, Applicants respectfully request that the rejection of claim 31 over Appelberg et al. under 35 USC § 103(a) be withdrawn and not be applied to the newly added or amended claims.

**CONCLUSION**

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. **643032000200**. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: April 11, 2008

Respectfully submitted,

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